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UROLOGIC TUMORS

Cytogenetics of the Progression of Adult Testicular Germ Cell Tumors

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INTRODUCTION

This article, written on the occasion of Dr. Avery Sandberg's 75th birthday, is intended to give a general and short overview of the cytogenetic data about testicular germ cell tumors of adolescents and adults we obtained from 1983 till now. The first and last author of this manuscript visited Dr. Sandberg's lab in 1984 to improve their skills in culturing and karyotyping solid tumor cells. Knowing that cancer and progression of cancer is caused by genetic changes (i.e. changes at the chromosomal or gene level), we investigated the karyotypes of about 140 testicular germ cell tumors of the adult and adolescent male (TGCTs). The chromosomal analyses of these tumors may shed light on oncogenesis, tumor progression, pathogenetic relationship, and therapy-related differentiation. We will focus on what we think our cytogenetic data have taught us about the progression of TGCTs. Of course, a way of thinking is always influenced or even determined by the results of others. This article is not intended as a review of the literature. For this the reader should consult a recent and excellent review by Sandberg et al. [1], the manuscripts of others, and our previous publications about germ cell tumors.

TESTICULAR GERM CELL TUMORS

Germ cell tumors of adult and adolescent males are rare neoplasms, located in the gonads (testis) and in extragonadal sites (e.g. retroperitoneum, mediastinum, and brain). Primary testicular germ cell tumors of adolescents and adults (TGCTs) can be divided clinically and morphologically

into two distinct entities [2,3]: seminomas (SEs), reflecting differentiation along the germ cell lineage, and nonseminomatous TGCTs or nonseminomas (NSs), of which pluripotent embryonal carcinoma (EC) cells are the stem cells. EC may differentiate into extraembryonic cell types resulting in choriocarcinoma (CH) and yolk sac tumor (YS) or along the lines of embryonic cells and tissues, resulting in immature teratoma (IT) and mature teratoma (MT). Most NSs have a mixed histology with the different histologic elements geographically separated or truly mixed. A minority of TGCTs contain both a SE and NS component, the combined tumors (CTs). Most TGCTs are thought to be derived from dysplastic germ cell precursors that progress to carcinoma in situ (CIS) [4]. It is suggested that the initiation of TGCTs starts early in life, probably before birth [5,6].

Whether and to what degree SEs and NSs are pathogenetically related is still a matter of debate. In essence, two models exist about the pathogenetic relationship between SEs and NSs [2,3,7–9]. In the first model, the histogenesis of SEs is assumed to diverge from that of NSs at an early stage. The neoplastic germ cells may either give rise to SE, reflecting germ cell differentiation or may differentiate into embryonic or extraembryonic tissues resulting in NS. According to this model the neoplastic pathways of SEs and NSs are different, with no or only limited crossover ([7], for review).

The second model suggests that SEs and NSs have a common origin with a single neoplastic pathway, in which SE may present an intermediate stage in development towards NS. According to this view, SE may either be an end stage in differentiation, or alternatively may progress to a NS phenotype. As a consequence, SEs and NSs may be closely related.

ONCOGENESIS AND PATHOGENESIS OF SEMINOMAS AND NONSEMINOMAS

To answer questions raised above about oncogenesis and pathogenesis, we investigated the karyotypes of five cases of CIS [10,11], 32 cases of SEs [12,13], 70 cases of NSs [12,14], and 31 cases of residual mature teratomas (RMTs) following chemotherapy [15,16]. Table 1 shows the number

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Table 1 Normal copies of chromosomes and i(12p) per case in CIS

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	i(12p)	N*
1	3	2	3	4	2	2	3	5	5	3	3	?4	3	2	5	2	2	3	1	?6	6	4	3	-	-	79
2	3	3	3	1	2	3	3	4	-	4	2	3	2	2	1	3	3	2	2	4	1	3	3	2	-	61
3	3	3	2	1	3	2	4	2	2	2	3	2	3	2	2	2	2	3	2	2	1	2	2	-	2	55
4	3	3	3	3	2	2	3	4	3	2	2	3	3	3	3	3	3	2	3	2	4	4	2	2	2	68
5	2	1	2	2	2	3	3	3	3	1	2	2	2	2	1	2	2	-	2	2	3	2	2	1	?1	?65
Total	14	12	13	11	11	12	16	18	13	12	12	14	13	11	12	12	12	10	10	16	15	15	12	5	5	

N* = modal chromosome number, structural abnormal chromosomes included.

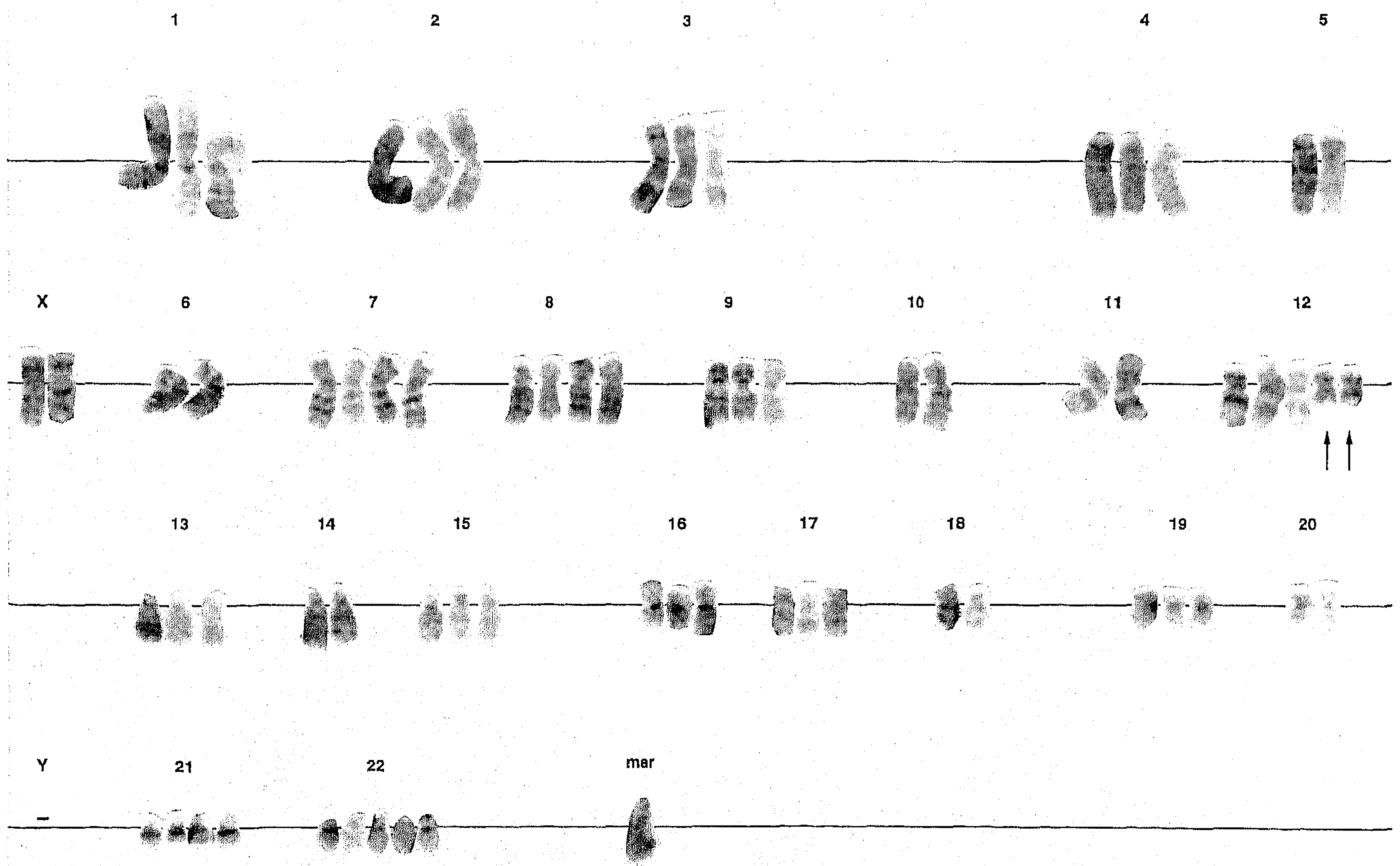
of normal copies of chromosomes, the number of i(12p) and the modal chromosome number of the 5 cases of CIS. The modal chromosome number ranged from 55 to 79; with an average of 66 [11]. In the 32 SEs the modal chromosome number ranged from 58 to 112 with an average of 73.4 and in the 70 NSs, from 50 to 113; with an average of 65.0 [12]. Figures 1, 2, and 3 show karyotypes of a case of CIS, SE, and NS, respectively.

For the SEs, NSs, and RMTs we determined for each tumor and chromosome the modal number of short and long arms. Parts of chromosomal arms involved in structural abnormalities were registered as whole arms if they represented 50% or more of the total arm length. The

modal number of short arms plus long arms divided by 2 revealed the average modal number of chromosomes. The average number of sex chromosomes for each tumor was multiplied by 2 to allow comparison with the autosomes [12–16].

Figure 4 shows the average modal number of short and long arms for each chromosome in the SEs, NSs, and RMTs. The average number of copies of the different chromosomes was highly similar in SEs and NSs (Spearman rank correlation 0.812, $P < 0.001$) [12]. We found both in SEs and NSs chromosome numbers in the triploid range with a significantly higher number of chromosomes in SEs than in NSs. These data are in agreement with ploidy studies

Figure 1 Representative karyotype of a case of CIS with the following karyotype description: 69,XX,-Y,-5,-6,+7,+8,-10,-11,+i(12)(p10)×2,-14,-18,-20,+21,+22,+22,+mar.



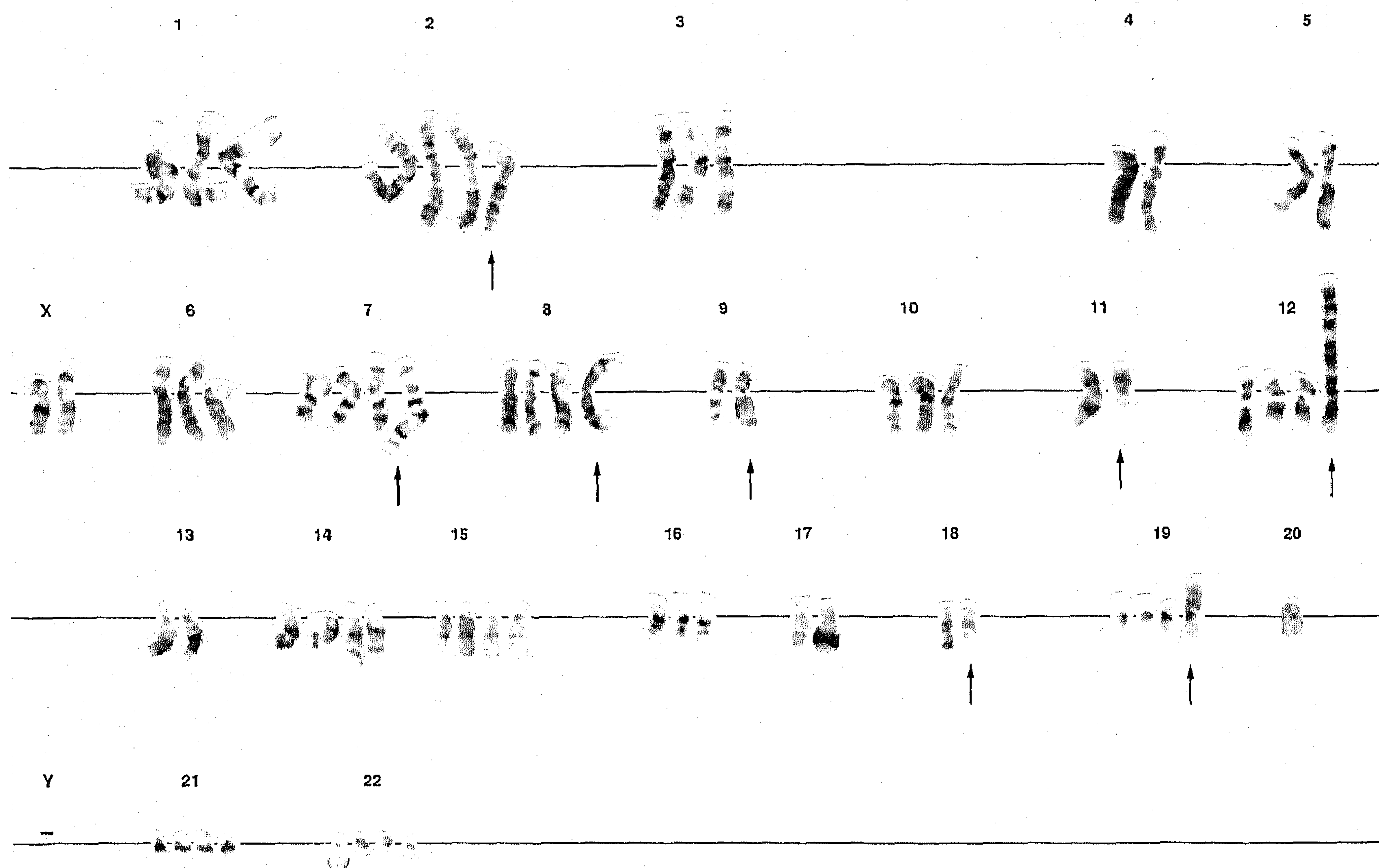


Figure 2 Representative karyotype of a case of SE with the following karyotype description: 68,XX,-Y,+add(2)(p11),-4,-5,+der(7)t(1;7)(q21;q32),+i(8)(q10),-9,der(9;17)(p10;q10),-11,add(11)(q13),+der(12)t(12;9)(p13;q12),-13,+14,+15,-17,-18,del(18)(q21),+der(19)t(9;19)(q12;p12),-20,-20,+21,+22.

on SEs and NSs, showing, on average, a higher DNA index (DI) in SE than in NS [8,17-20]. We found the median DI of CIS about the same as that of SEs and higher than the DI of invasive NSs [19]. Together with our finding of a very similar distribution pattern of the different chromosomes in SEs and NSs (Fig. 4) as well as the resemblance in distribution of breakpoints in SEs and NSs [12] these data fit with a pathogenetic model of TGCTs suggesting that SEs and NSs have a common origin with a single neoplastic pathway, with SE constituting an intermediate stage in NS development [8,9,12,19]. This view is supported by the cytogenetics of a CT we karyotyped. Of three cases of combined tumors (CTs) we karyotyped the SE and NS component separately [21]. In one case the SE and NS component of the CT shared eight different structural chromosomal abnormalities, indicating that in this tumor the SE and NS component are pathologically closely related and have a common neoplastic pathway for a considerable length. Recently we obtained supportive data for the hypothesis of a common origin of SEs and NSs using in situ hybridization with region specific probes on tissue sections [22].

Our cytogenetic data of TGCTs support the linear progression model [7-9, 12] in which SE may be an end stage in differentiation as well as an intermediate stage in the development of CIS to NS. Both SEs and NSs are supposed

to be derived from CIS [4]. We described the similarities in chromosomal pattern between two cases of TGCTs and its adjacent CIS [11]. These results represent cytogenetic evidence that CIS is clonally related to, and is the precursor for, invasive TGCTs. This has been supported using in situ hybridization on tissue sections [22,23].

An important and early event in the oncogenesis of TGCTs is polyploidization of a dysplastic germ cell precursor, resulting in aneuploid CIS. The progression of CIS → SE → NS is accompanied by net loss of chromosomes [8,9,12,19]. The CIS karyotypes (Table 1) revealed some evidence for karyotype evolution. If polyploidization is a very early oncogenetic step one would expect to find, in the absence of karyotype evolution, similar numbers of the different chromosomes. This is not the case [10,11]. Because the number of cases of CIS and the number of analyzed metaphases is small, it is rather speculative to derive definite conclusions regarding the chromosomal constitution of CIS. But it is remarkable that some chromosomes, overrepresented in SEs and NSs, already show a trend of overrepresentation (e.g. chromosomes 7 and 8) in CIS.

i(12p) is the characteristic chromosomal abnormality of TGCTs [24]. We found i(12p) in about half of our karyotyped cases of CIS (Table 1). This suggests that i(12p) formation is an important and early event in the oncogenesis of

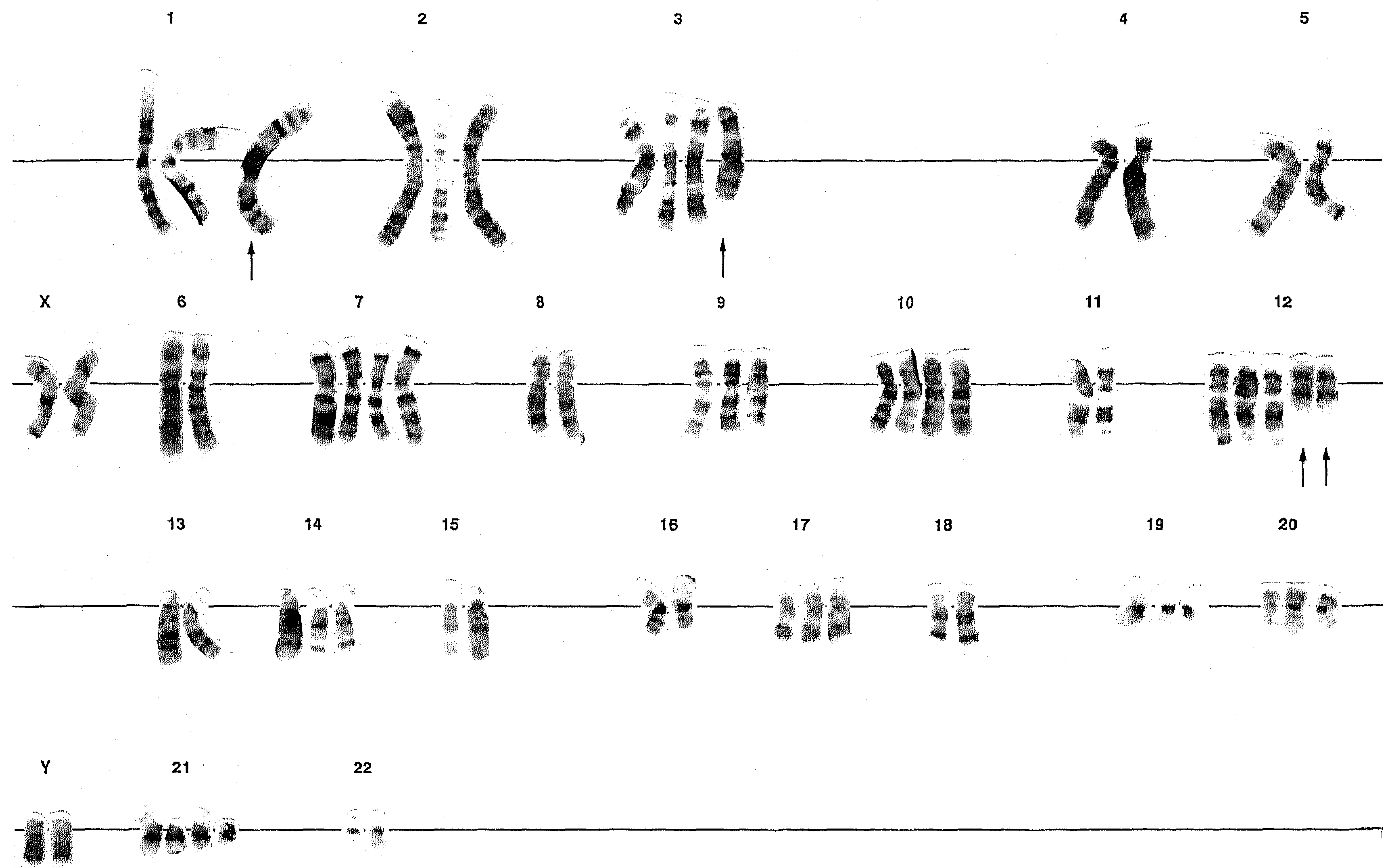


Figure 3 Representative karyotype of a case of NS with the following karyotype description: 66,XXY, +Y,i(1)(q10),+der(3)t(3;8)(q23;q22),-4,-5,-6,+7,-8,+10,-11,+i(12)(p10)×2,-13,-15,-16,-18,+21,-22.

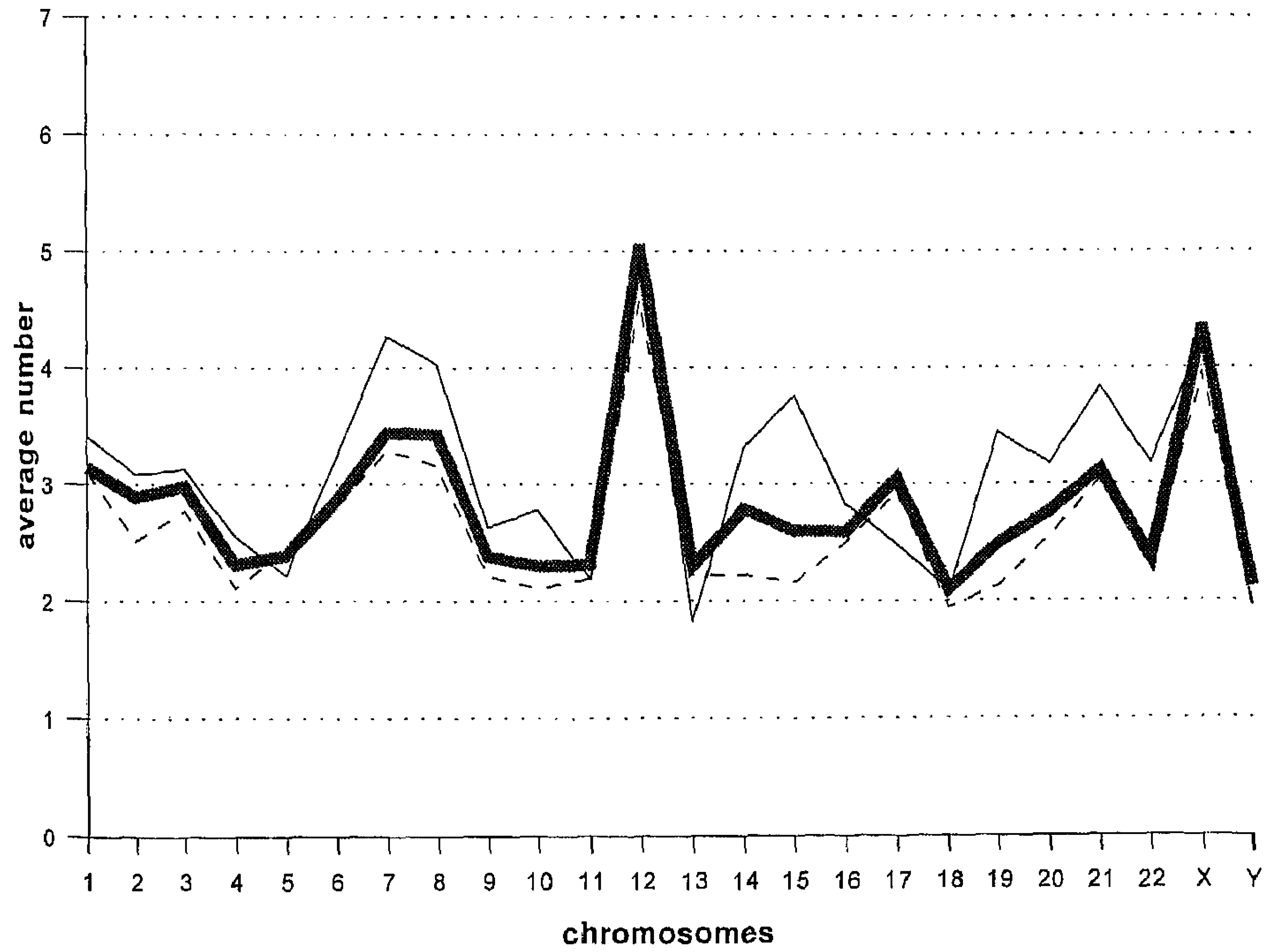
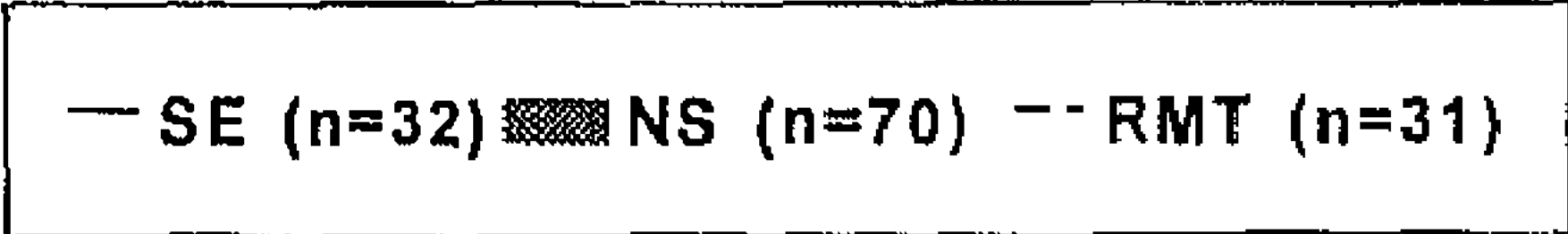


Figure 4 Average modal number of short and long arms per chromosome in a group of 32 SEs (thin line), 70 NSs (dark line), and 31 RMTs (dotted line). The average number of sex chromosomes for each case was multiplied by 2 to allow comparison with the autosomes.

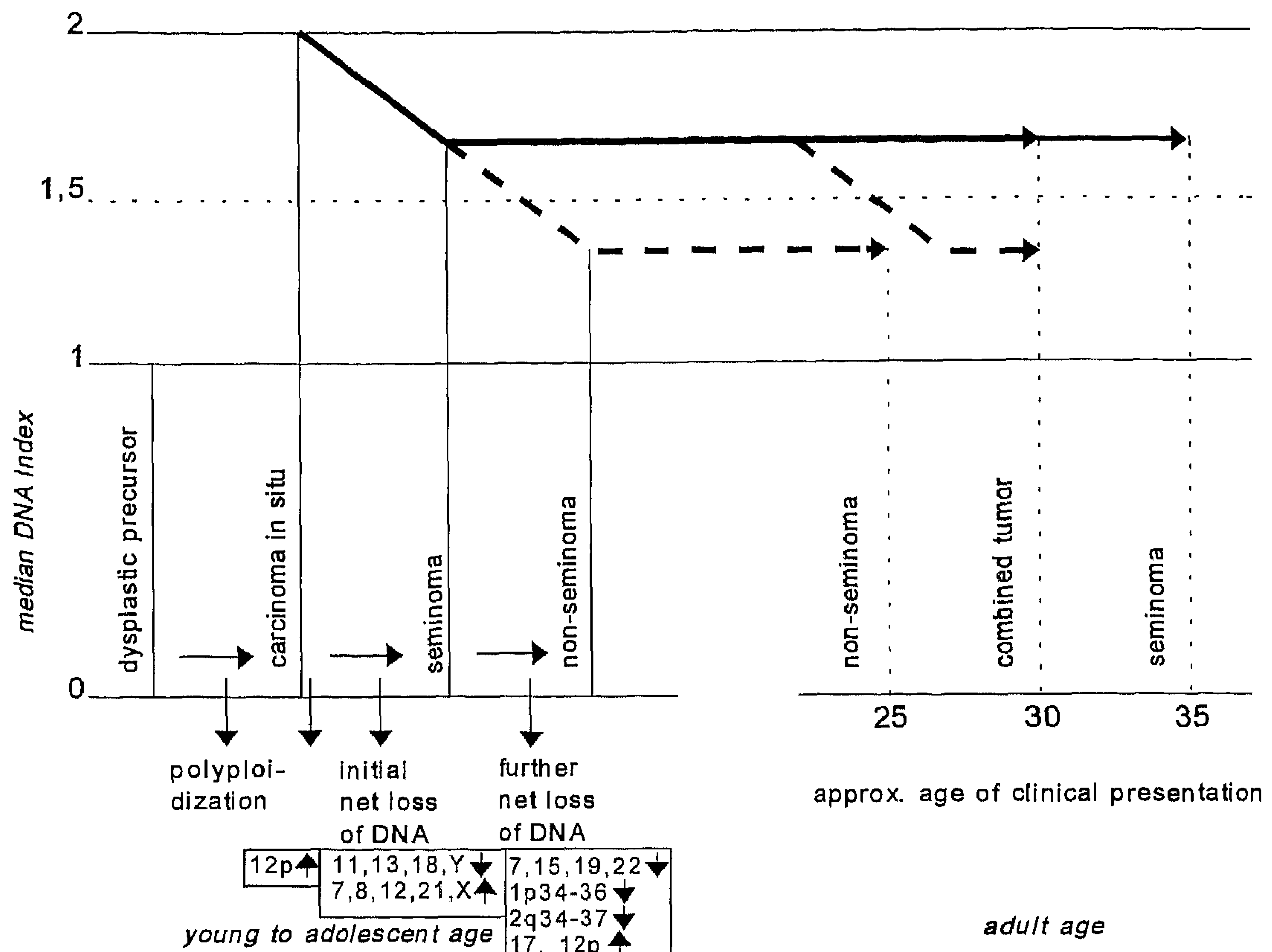


Figure 5 Tumor progression model of TGCTs of adults proposed by Oosterhuis et al. [8], supplemented with our relevant chromosomal data.

TGCTs, although most likely preceded by polyploidization [25]. Both the frequency and the number of copies of *i*(12p) are higher in NSs than in SEs [12]. We found *i*(12p) in 56% of the SEs and in 83% of the NSs. The average number of copies in the SEs was 0.9 and in the NSs 1.7. When the linear progression model in which SE may be an end stage in differentiation as well as an intermediate stage in the development of CIS to NS [7-9] is correct this might indicate that an increase in *i*(12p) copy number may be related to tumor progression from SE to NS. We and others observed aberrations of chromosome 12 in *i*(12p)-negative TGCTs [12,26-28]. FISH studies have shown that *i*(12p)-negative tumors consistently show amplification of 12p [29,30]. Our cytogenetic data extended with our molecular data point to an overrepresentation of the region 12p11.1-p12.1 [31,32]. The consistent overrepresentation of 12p sequences, by *i*(12p)-formation or other aberrations of chromosome 12, indicates that a gene on 12p plays an important role in the oncogenesis of TGCTs. However, the identification of this gene remains to be established.

The gain and loss of chromosomes during the progression of TGCTs, resulting in net loss of chromosomal material, is nonrandom; specific chromosomes are overrepresented (e.g. 7, 8, 12, 21, and X), and others are underrepresented (e.g. 11, 13, 18, and Y) (Fig. 4)[9, 12-16]. Although our series of CIS is small, we have indications that losses and gains of chromosomes already occur in CIS [9-11]. In situ

hybridization [23] and DNA flow cytometry studies [33] also point to karyotype evolution in CIS. The autosomes that are overrepresented in TGCTs may harbor growth promoting genes and relevant tumor suppressor genes may be located on the underrepresented chromosomes. In SEs, a significantly higher copy number of chromosomes 7, 15, 19, and 22 was found and a significantly lower copy number of chromosome 17, compared with NSs. Besides these numerical chromosomal differences we also observed some significant differences in structural chromosomal abnormalities between SEs and NSs [12]. These chromosomal differences between SEs and NSs may play a role in tumor progression or in the direction of differentiation of SE and NS [8,9,12]. Specific over- and underrepresentation of (parts of) chromosomes as detected by conventional karyotyping in TGCTs has recently been verified by using comparative genomic hybridization [32].

In conclusion, important steps in the oncogenesis or progression of TGCTs are polyploidization, overrepresentation of 12p sequences, overrepresentation of some specific chromosomes or parts of chromosomes, and underrepresentation or retention of others, resulting in net loss of (parts) of chromosomes. This multistep process of polyploidization, overrepresentation of 12p sequences and specific gain, loss, or retention of chromosomes and parts of chromosomes results in a more or less characteristic chromosomal pattern for TGCTs (Fig. 4). Figure 5 shows the tumor

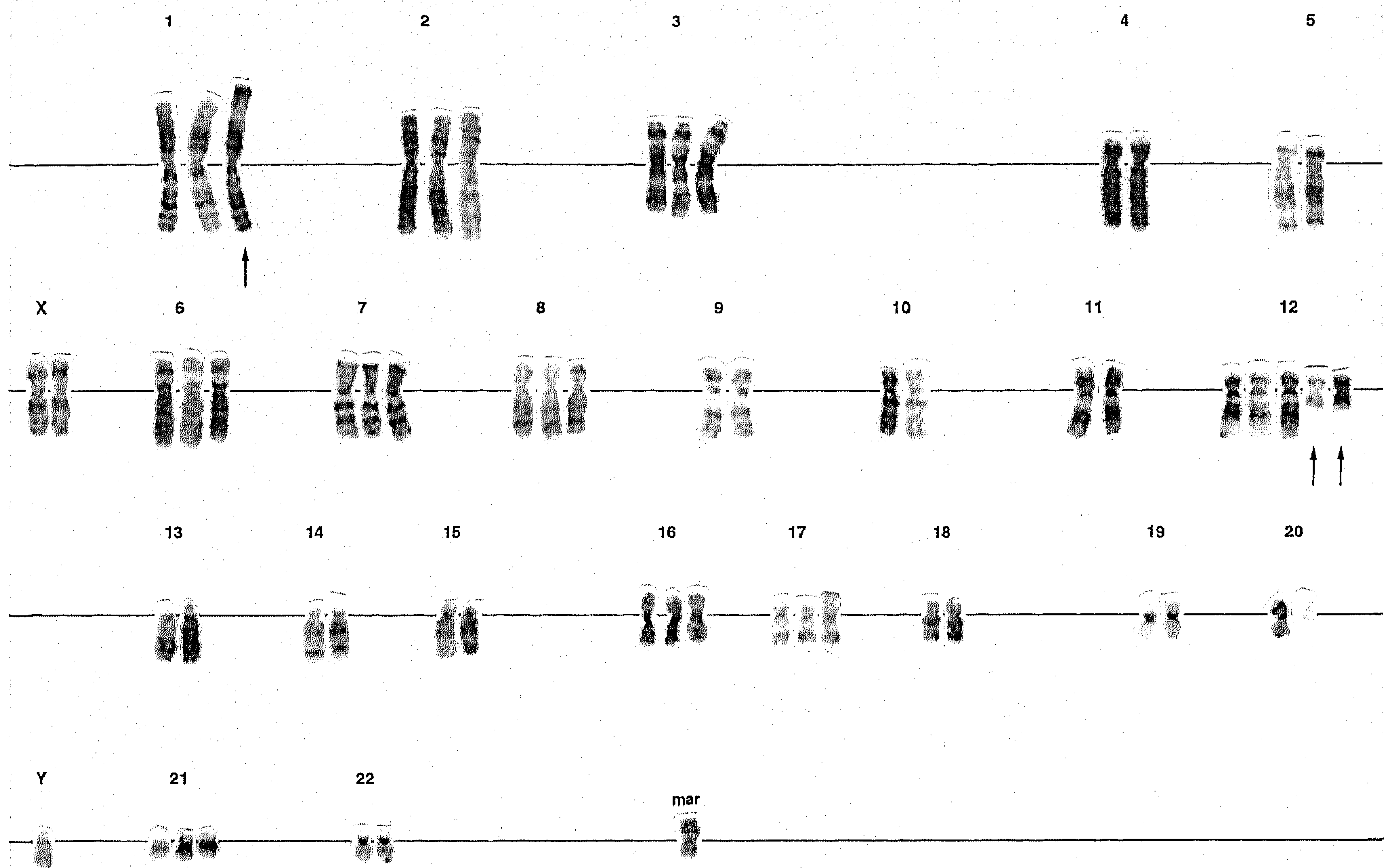


Figure 6 Representative karyotype of a case of RMT with the following karyotype description: 60,XXY, add(1)(p34),-4,-5,-9,-10,-11,+i(12)(p10)×2,-13,-14,-15,-18,-19,-20,-22,+mar.

progression model of TGCTs of adults proposed by Oosterhuis et al [8], supplemented with our relevant cytogenetic data [12].

i(12p)-formation and over- and underrepresentation of chromosomes already occur in CIS. This may suggest that karyotype evolution/tumor progression in TGCTs already takes place in an early stage of tumor evolution. To substantiate this notion, it would be of importance to obtain more cytogenetic data of CIS adjacent to invasive tumor and of CIS before it has progressed to invasiveness. Comparison of the different stages of CIS with invasive tumor may shed further light on the process of tumor evolution in TGCTs.

METASTASIS AND THERAPY-RELATED DIFFERENTIATION OF TGCT METASTASES

Cytogenetic comparison of primary tumors and metastases may indicate chromosomal changes playing a role in tumor progression. Tumor progression is the result of clonal evolution of a tumor cell population and is paralleled by karyotype evolution [34]. Because of clonal evolution and selection, malignant tumors are genetically heterogeneous and contain multiple subpopulations of cancer cells. Only certain subpopulations of tumor cells have the capacity to form metastatic lesions [35,36].

Because of the application of standardized clinical protocols, we are not able to investigate the chromosomal pattern of untreated metastases of NSs. It is only possible to study residual lesions following chemotherapy, usually RMT. These lesions are composed of fully differentiated tissue [37]. This higher degree of differentiation after chemotherapy treatment might be because of direct induction of differentiation of tumor cells to fully differentiated cells, to selective destruction of cells other than MT cells, or to selection of cells with an inherent capacity of spontaneous differentiation or capacity of therapy related differentiation ([2,37-39], for review).

A cytogenetic comparison between NSs and RMTs may shed light on which chromosomal changes play a role in tumor progression and on the mechanism(s) of therapy related differentiation, although a distinction between both events cannot be made.

We karyotyped 31 RMTs [15,16]. Figure 6 shows one of the karyotypes. The average modal total chromosome number was 60.5. That all tumor cells from RMT are aneuploid was demonstrated by using a dual parameter flow cytometric analysis [40]. We found in 81% of the RMTs an i(12p) chromosome with an average copy number of 1.5. In NSs and RMTs the total modal chromosome number does not differ. The median DNA index of primary NSs and lymph node metastases are in the same range [20].

The average number of copies of the different chromosomes and parts of chromosomes are highly similar in the NSs and RMTs (Spearman rank correlation 0.918, $P < 0.001$) (Fig. 4), both the frequency and average copy number of i(12p) are in the same range and the distribution of chromosomal breakpoints does not differ significantly in both series [15].

So our cytogenetic comparison between the series of 70 NSs and 31 RMTs revealed no significant chromosomal differences between the primary tumors and the metastases after chemotherapy. This may be explained in different ways [15]:

1. In vitro selection. Metastases result from the selective growth of specialized subpopulations of cells of the primary tumor [35,36]. Culturing the heterogeneous primary tumor may select for cells of the specific subpopulations that finally populate the metastases. Under the influence of therapy the metastatic cells differentiate irrespective of their highly abnormal karyotype.
2. Clonal dominance. The progeny of a single metastatic clone could eventually overgrow the primary tumor. As a result late-stage advanced primary tumors would be biologically similar to distant metastases [41]. Both may show similar karyotypes. Under the influence of therapy the metastatic cells differentiate irrespective of their highly abnormal karyotype.
3. One would not expect to find chromosomal differences between primary NSs and RMTs, when metastasis is not paralleled by visible chromosomal alterations and when RMTs are the result of therapy-related induction of differentiation of cells, irrespective of their chromosomal pattern.

The chemotherapy related differentiation of RMTs might be because of selective destruction of cells other than MT or to selection of other cells with an inherent capacity of (therapy related) differentiation. If, however, RMTs are the result of differentiation of selected cells with a chromosomal constitution allowing differentiation one would only expect to find specific chromosomal differences between primary NSs and RMTs when the direction or degree of differentiation within NSs is paralleled by visible chromosomal changes. We never found evidence for this [42, 43].

In conclusion, primary NSs and metastatic mature testicular lesions left behind after chemotherapy have comparable chromosomal patterns. We found no cytogenetic evidence that specific chromosomal changes parallel metastasis and therapy related differentiation of metastases. Based on cytogenetics, both induction of differentiation of (selected) cells or selection of cells with capacity to differentiate are possible mechanisms for the therapy related differentiation of RMTs.

TGCTs do not have one single or only a few specific chromosomal abnormalities. They show a highly abnormal, more or less characteristic, chromosomal pattern. This pattern results from the accumulation of a restricted scale of chromosomal abnormalities that enables the development, sur-

vival, and progression of TGCTs. Deviation from this pathway leads to a chromosomal pattern not compatible with (further) development as a TGCT. The highly restricted pattern of chromosomal abnormalities may explain our finding of identical or almost identical karyotypes in recurrent lesions, compared to primary tumors, in the same patient even after several years [44].

Despite their benign histologic appearance RMTs have a highly abnormal karyotype not distinguishable from that of primary NSs. Thus RMTs should be considered as wolves in sheep clothing, which justifies their complete surgical removal [45].

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